BMEM Protein-Free Cardiomyocyte Differentiation Kit – optimized cardiomyocyte differentiation for human iPSC

Ships at room temperature

Catalog number: 1003 **1 kit**

Description

BMEM Protein-Free Cardiomyocyte Differentiation Kit is a ready-to-use highly optimized, chemically defined, animal component-free kit for the differentiation of human induced pluripotent stem cells (hiPSC) to contracting cardiomyocytes (hiPSC-CM) in adherent culture.

This kit is a novel formulation optimized without any proteins. Differentiation using this kit typically produces contracting cardiomyocytes in 8-9 days and results in ~85-90% $TNNT2^{+}$ cells and yields of $>1x10^{5}$ cells per cm^{2}. Cardiomyocytes can be maintained in BMEM Cardiomyocyte Maintenance Medium for >45 days.

BMEM Protein-Free Cardiomyocyte Differentiation Kit is compatible with hiPSC growth medium BMEM Human Primed Pluripotent (1001).

BMEM Protein-Free Cardiomyocyte Differentiation Kit is compatible with a variety of culture matrices including: Corning® Matrigel® GFR (354230), Gibco™ Geltrex™ GFR (A1413202), R&D Systems™ Cultrex™ UltiMatrix GFR (BME001-05), Gibco™ Recombinant Vitronectin (A14700), and Corning® Synthemax II-SC (3535).

Each lot of BMEM Protein-Free Cardiomyocyte Differentiation Kit is performance tested on hiPSC and sterility tested

COMPONENT NAME	SIZE	STORAGE
BMEM Cardiomyocyte d0	100 mL	Store at 4 °C
BMEM Cardiomyocyte d1	100 mL	Store at 4 °C
BMEM Cardiomyocyte d2	100 mL	Store at 4 °C
BMEM Cardiomyocyte Maintenance Medium	500 mL	Store at 4 °C

Instructions for Use

BMEM Protein-Free Cardiomyocyte Differentiation Kit is ready to use. Bottles can be used straight from the refrigerator (4 °C) and should not be warmed to RT or 37 °C prior to use.

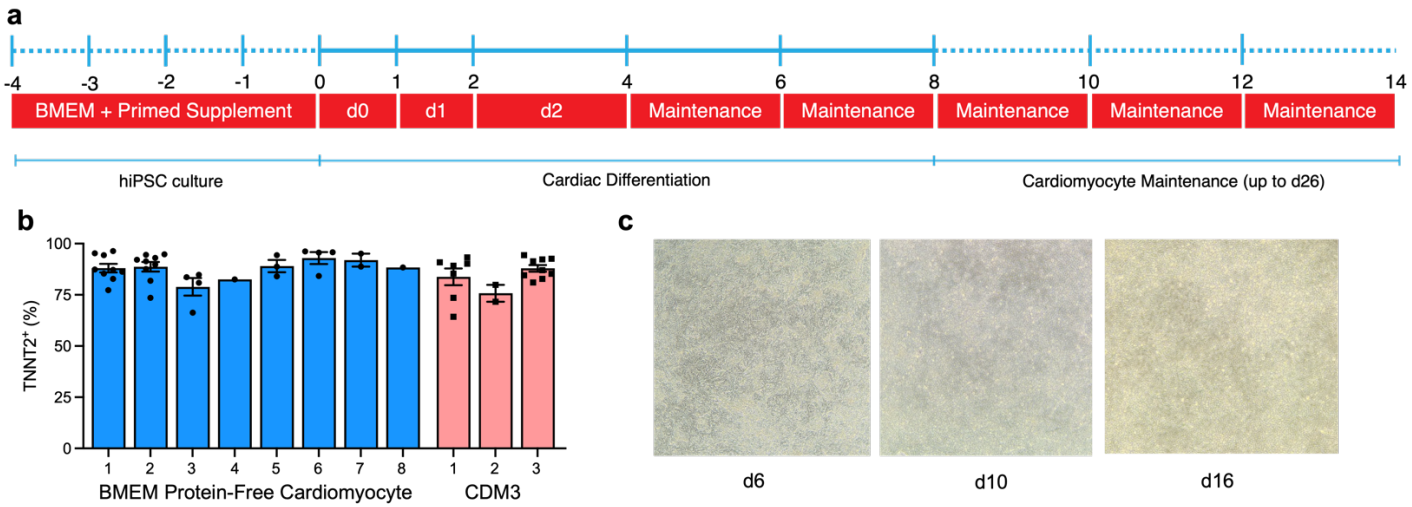
Directions for Use

hiPSC should be growing on a consistent schedule achieving 70-80% confluence before differentiation. Fast, consistent growth, without overgrowth (>90% confluence) is essential for reproducible differentiation. We recommend that hiPSC lines are ≥p20 for reproducible differentiation. For consistency, media changes should be completed at the same time of day.

1. Grow hiPSCs to 70-80% confluence, typically this takes 4 days.
2. Aspirate pluripotent media and replace with BMEM Cardiomyocyte d0.
3. After 24 hours aspirate media and replace with BMEM Cardiomyocyte d1.
4. After 24 hours aspirate media and replace with BMEM Cardiomyocyte d2.
5. After 48 hours aspirate media and replace with BMEM Cardiomyocyte Maintenance Media.
6. Every 48 hours aspirate media and replace with BMEM Cardiomyocyte Maintenance Media.

Contracting cardiomyocytes should be seen after 8-9 days.

We recommend replating hiPSC-CMs to new wells for assays between d16 and d20.



Product Use

For Research Use Only.

Not for diagnostic procedures.

Troubleshooting

1. High levels of cell death on d0-d1.
    Cause: hiPSC were over (>90%) or under (<50%) confluent prior to starting differentiation.
        Solution: Adjust split ratio of pluripotent cells to achieve 70-80% confluency. Keep culture conditions consistent.

2. Full contracting monolayer is not achieved.
    Cause: hiPSC were under (<60%) or over (>90%) confluent prior to starting differentiation.
        Solution: Adjust split ratio of pluripotent cells to achieve 70-80% confluency. Keep culture conditions consistent.

3. No contracting cells by d10.
    Cause 1: media change schedule deviated too far from prescribed protocol, potentially with inadequate exposure time to d0 media.
        Solution: Make sure that media changes accurately follow the described schedule: 24 h d0, 24 h d1, 48 h d2.
    Cause 2: hiPSC are growing poorly.
        Solution: Grow cells until a reproducible split ratio every 4 days, as described above, is achieved.
    Cause 3: Media is too old.
        Solution: Media should be used within 30 days of opening.

4. Contracting monolayer constantly detaches from the plate surface.
    Cause: extracellular matrix is not suitable for long-term maintenance of hiPSC-CMs without passage, such as vitronectin or Synthemax.
        Solution: Use an alternative matrix. If matrices such as vitronectin are desired to be used, then hiPSC-CMs should be passaged at d8-d10 when they can be replated into vitronectin.